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The Influence of MMP-3 towards MMP-9 among Emphysematous Patients from Gingival Crevicular Fluid and Sputum

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Abstract
The elevated activity of matrix metalloproteinase (MMP)-9 has been responsible for degradation of extracellular matrix (ECM) within lung parenchyma leading to emphysema among patients suffering from chronic obstructive pulmonary disease (COPD). Prolonged exposure of smoking had triggered activation of both MMP-9 and MMP-3. Active MMP-3 might degrade numerous kinds of ECM and act as MMP-9 inducer as well. The study aimed to correlate active MMP-3 towards elevated MMP-9 activity from both gingival crevicular fluid (GCF) and sputum to assess breakage of extracellular matrix (ECM) among emphysematous patients. Fifteen emphysematous respondents suffering from COPD were recruited to undergo thoroughly physical assessment, spirometry, and radiological examination. Then, both GCF and sputum were collected for measurement of MMP-3 and MMP-9 activity. Results showed that MMP-3 activities were correlated positively and significant with elevated MMP-9 activities from both GCF and sputum i.e. r = 0.899 (p<0.05) and r = 0.770 (p<0.05) respectively. Smoking exposure released many radicals and oxidants generating elevation of MMP-3 activity which then influenced repeatedly influx of neutrophils and activation of MMP-9. The role of active MMP-3 also involved in either acute inflammatory or broad ECM breakage. Moreover, active MMP-9 might lead mainly the degradation of ECM within lung parenchyma. Because of similar effect and impact concerning ECM degradation, both active MMP-3 and MMP-9 might concurrently cause larger breakage of ECM leading to lung emphysema among COPD patients. This study showed that both GCF and sputum would be assigned to evaluate active MMP-3 and MMP-9 for assessing ECM degradation among emphysematous patients.
Keywords: MMP-3, MMP-9, GCF and sputum, influence, emphysematous

Introduction

Smoking has been the major risk factor for COPD development of 20-25% smokers. The more cigarette consumed is linear with the higher risk occurs among smokers[1]. Smoking also exposes oral cavity including the periodontal tissues (Pejčić *et al.*, 2007; Usher AK and Stockley RA, 2013). Smoking induces inflammatory mediators leading releasing neutrophils, macrophages, and other cytokines from exposed tissues. Within lung, prolonged exposure of smoking might lead the morphological changes namely emphysema. The disorders of alveoli, enlargement of terminal bronchi, decrease of elastic recoil, and worse lung function are easily found among emphysematous patients (Daheshia, 2005; Churg *et al.*, 2012).

Emphysema mostly happens as elastolytic destruction process since Matrix metalloproteinase (MMP)-9 activity increases and so does MMP-9/TIMP-1 ratio (Churg A *et al.*, 2012; Le Quément *et al.*, 2008). Evaluation of both MMP-9 activity and MMP-9/TIMP-1 ratio has been useful to assess the progressivity of emphysema among chronic obstructive pulmonary disease (COPD) (Boschetto *et al.*, 2006). Those parameters also take place in destructing periodontal tissues (Rai *et al.*, 2010). Some studies still engaged MMP-9 activity to assess the recovery of COPD and periodontitis among patients who stop smoking after treatment as MMP-9 activity has shown elevated and influenced poor recovery of diseases (Louhelainen *et al.*, 2010; Rauten *et al.*, 2011).

Regarding MMP-3, it has numerous substrates, as does MMP-9. Moreover, MMP-3 is able to activate MMP-9 (Lagente *et al.*, 2005). Normally, the active MMP-9 is bound tightly by TIMP-1 which inactivates MMP-9. Nevertheless, the active MMP-3 might activate pro MMP-9 to become active MMP-9 which next vulnerably degrade extracellular matrix of smoking-exposed tissues particularly lung and periodontium (Dahlen *et al.*, 1999; Ziora *et al.*, 2008).

To date, biomarkers have been developed in order to assess the severity of disease based on cytokines, chemokines, oxidants and proteases. They were apparently helpful to evaluate pathophysiology, inflammation stages, destruction and lung remodeling in COPD. But, the availability of opted biomarkers should be considered by using minimally invasive procedures. The study suggested gingival crevicular fluid (GCF) and sputum as they were easily collected for assessing both MMP-3 and MMP-9 from lung and periodontium due to smoking (Snell N and Newbold, 2008; Cazzola M and Novelli, 2010).

The study aimed to correlate the influence of active MMP-3 towards active MMP-9 from both GCF and sputum among emphysematous patients.

Materials and Methods

Procedure

All The study methods and procedures were approved officially by the Ethical Committee of Faculty of Medicine, Syiah Kuala University, Banda Aceh-Indonesia. Fifteen outpatients at Pulmonary Policlinic of Zainoel Abidin Hospital, Banda Aceh-Indonesia were recruited as respondents. They were male, >50 year olds, smokers or ex smokers, had 20 pack years and more. Those with asthmatic, infection of respiratory lower tract and pulmonary malignancies were excluded as respondents.

Furthermore, all eligible respondents had to undergo the physical examination, spirometry test, and CT scan as well. And showed FEV_1/FVC ratio <70%, FEV_1% predicted <50%. Next, respondents underwent sputum induction by nebulizer-assisted 3% NaCl solution collected into sterile pots. Then, GCF was collected by putting a small piece of cut *Whatman*® filter paper (2 mm x 8 mm) into gingival sulci stored into micro-centrifuge tubes (Johnson *et al.*, 1999). All samples were preserved into 80^0C freezer for further analysis. The activity of MMP-9 from both sputum and GCF was analyzed by using *Sensolyte® 520 Generic MMP assay kit Fluorimetric*.

Results and Discussion

Characteristics of Respondents and MMP-3, -9 Activities

Respondents were patients suffering from emphysema who had means (SD) of age and FEV_1/FVC ratio were 59.53 (5.579) years old and 44.65 (7.356) % respectively. Regarding MMP-3 activities, they were 1.36 (0.892) μM and 1.70 (1.330) μM from GCF and sputum respectively. Moreover, the MMP-9 activities were 0.94 (0.722) μM and 1.65 (1.574) μM from GCF and sputum respectively. The data were as shown in Table 1.

Table 1. Characteristics of Respondents and MMP-3, -9 Activity

Variables	n	Mean (SD)
Age (yr)	15	59.53 (5.579)
FEV_1/FVC ratio (%)	15	44.65 (7.356)
MMP-3 Activity of GCF (μM)	15	1.36 (0,892)
MMP-9 Activity of GCF (μM)	15	0.94 (0.722)
MMP-3 Activity of sputum (μM)	15	1.70 (1.330)
MMP-9 Activity of sputum (μM)	15	1.65 (1.574)

Correlations of MMP-3 Activity towards MMP-9 Activity

The MMP-3 activities showed strong correlation towards MMP-9 activities from both GCF and sputum, i.e r=0.899 (table 2.) and r=0.770 (table.3) respectively.

Table 2. The Correlation of MMP-3 Activity towards MMP-9 Activity from GCF

	MMP-9 Activity	
	r	*p* value
MMP-3 Activity	0.899	<0.05

Table 3. The Correlation of MMP-3 Activity towards MMP-9 Activity from Sputum

	MMP-9 Activity	
	r	*p* value
MMP-3 Activity	0.770	<0.05

Our study chose gingival crevicular fluid (GCF) and sputum for evaluating MMP-3 and MMP-9 as they were easily collected with simple procedure. Previous studies have assessed the level and activity of MMP-9 from various body fluids, e.g. bronchoalveolar lavage (BAL) and blood serum. Increased activity of MMP-9 may cleave ECM of lung tissue. Normally, those suffering from COPD, having lower FEV_1 and FEV_1/FVC ratio <70, would also exhibit increased level and activity of MMP-3 and MMP-9. However, the study assessed activity of those proteases instead of level since activity assessment might represent the breakage of ECM. Activity has only measured active MMP-3 and MMP-9 cleaving substrates (Lowrey *et al.*, 2008).

The correlation of active MMP-3 towards active MMP-9 showed better correlation. Active MMP-3 is positively correlated with activity of MMP-9, i.e. r=0.899 (p<0.05) and r=0.770 (p<0.05) from GCF and sputum respectively. Regarding MMP-3, the fibroblast has stimulated pro inflammatory mediators, mainly TNF-ɑ, to release pro MMP-3. The activation of MMP-3 might be done by cathepsin G and elastase. The active MMP-3 could activate pro MMP-9. Then, both active MMP-3 and MMP-9 could cleave ECM concurrently (Beklen *et al.*, 2006).

The imbalance of protease and anti protease normally increased among COPD respondents found in sputum. However, Correlation of MMP-9 activity is linear with the number of neutrophils from inflamed airways caused by prolonged exposure of smoking leading ECM degradation of lung parenchyma commonly known as emphysema. Moreover, airway obstruction commonly occurs in small airway and lung parenchyma evaluated with radiological examination and spirometry (Boschetto *et al.*, 20006; Chaudhuri *et al.*, 2013).

The lung responded more than the periodontal tissues in regards with the activities of MMP-3 and MMP-9 among smokers with chronic obstructive pulmonary disease (COPD). Smoking exposure induced the raise of netrophils, macrophages, and other inflammatory mediators leading the release of MMP-3 and MMP-9. The excess of active MMP-3 and MMP-9 could degrade the extracellular matrix (ECM) of respective tissues, i.e. lung and periodontium. Besides ECM degradation, active MMP-3 was able to activate pro MMP-9. The active MMP-3 was related with increased expression of mRNA of MMP-3. Increased activity of MMP-3 was also influential with recovery process of injured tissues. The study has found the correlation of active MMP-3 and active MMP-9 with significantly strong correlation. Additionally, active MMP-3 could impair the balance between MMP-9 and TIMP-1 (Ziora *et al.*, 2008; Kumar, *et al.*, 2013).

The tide of MMP-9 activity apparently showed dynamic sequence relying on prolonged smoking exposure which was supposed to be responsible upon MMP-9 activity (Boschetto *et al.*, 2006). The active MMP-3 was supposedly involved in recruiting neutrophils influx into the tissues so that it enabled more severity and mortality to take place inside the tissues. The active MMP-3 was also responsible in acute stage of inflammation. Together with MMP-9, the degradation of extracellular matrix of tissues might end result worse (Nerusu *et al.*, 2007). Moreover, TIMP-1 also keeps arise to maintain the balance. Increased activity of MMP-9 corresponds with airway obstruction and destruction of extracellular matrix among COPD patients. Moreover, airway obstruction is also together with increase of MMP-9/TIMP-1 ratio (Abdella *et al.*, 2015).

Conclusions

The active of MMP-3 might be influential to activate MMP-9 from both GCF and sputum. Then, MMP-9 could raise its activity and supposedly lead to ECM degradation.

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